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Bringing Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging to the Clinics

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INTRODUCTION

Mass spectrometry imaging (MSI) is an emerging set of analytical techniques that hold promise in changing pathology and tissue-based diagnostics.[1] In its simplest sense, MSI is an analytical chemistry application in which a target is analyzed using a mass spectrometer in at least 2 spatial dimensions and, in doing so, preserves the relative spatial location of molecules during analysis. The net effect is the creation of a biochemical map or ion image, or, more precisely, hundreds to thousands of concomitant ion images, each of which corresponds to the spatial distribution of a particular ion. In clinical applications, MSI can be used to map both endogenous and exogenous small molecules,[2] lipids,[3] as well as peptides and proteins,[4] depending on the ionization technique and mass analyzer used. Several ionization techniques have been described, including secondary ionization mass spectrometry (SIMS) and ambient electrospray ionization such as desorption electrospray ionization (DESI), although these are outside of the scope of the review and are reviewed elsewhere.[5] This article focuses on MSI applications that use matrix-assisted laser ionization/desorption (MALDI) as the ionization source.

Although there are many variations in sample preparation and analysis in MALDI MSI, the overall approach is generally similar. MALDI MSI begins with mounting a specimen, such as a cryosectioned tissue, onto a conductive slide or plate. A chemical matrix, commonly a small organic acid such as α-cyano-4-hydroxycinnamic acid (α-CHCA) or

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2,5-dihydroxybenzoic acid (DHB), is applied to the surface either through spotting or spraying a solvent containing that matrix or by subliming the matrix onto the specimen. The matrix is then dried, the sample is moved to the source and placed under vacuum, and a region of interest is selected for analysis. Analysis is performed by firing a laser at the surface that desorbs ions from the tissue, which are then analyzed by the mass spectrometer. The most common mass spectrometers used in MALDI MSI are time of flight (TOF) mass analyzers. For certain applications, high-resolution mass spectrometers such as Fourier transform ion cyclotron resonance (FT-ICR) MS provide more robust and specific analysis. More recently, ion mobility has been added to these instruments to enhance their analytical power. Although MALDI MSI was described more than 20 years ago,[6] it is in the last few years that significant inroads into the translational and clinical space were made. This article provides a brief overview on clinical workflows; reviews clinical opportunities to use MALDI MSI, such as neoplastic and infectious diseases; and reviews some analytical parameters to consider when thinking about these applications.

CLINICAL WORKFLOWS AND OPPORTUNITIES

Before discussing MALDI MSI applications, it is valuable to provide the setting and clinical workflows in which these different applications can be used. There are no universal clinical settings, because there are differences between smaller community hospitals, academic centers, and private practices as well as regional and international differences. However, this is discussed in a general sense, with additional details as pertains to our own setting working in an academic medical center. In a broad sense, most clinical specimens are analyzed by pathologists in either clinical pathology (CP) or anatomic pathology (AP). AP largely consists of analysis of tissue specimens, and is often subdivided into surgical pathology, cytopathology, and autopsy pathology, and can be further divided into subspecialized areas focused on different tissues or organs. AP covers almost all clinical tissue specimens and is therefore the primary area for MSI-based approaches. In contrast, CP, also known as laboratory medicine, largely involves the analysis of liquid specimens, such as blood, urine, cerebrospinal fluid, and other body fluids, and is further divided into chemistry, hematology, microbiology, molecular pathology, and blood banking/transfusion medicine. There are tissue specimens such as lymph nodes and bone marrow biopsies that are handled by hematopathologists and other areas of overlap. For simplicity, the focus in this review is on areas traditionally overseen in AP.

Most tissue specimens are obtained through surgical excisions or biopsies in the operating room or through certain outpatient procedures such as colonoscopies. In certain cases, the surgeon collects a small representative section for intraoperative analysis, or the so-called frozen section procedure,[7] in which a tissue sample is brought to the pathology frozen section room, where it is rapidly frozen in an embedding medium, sectioned using a cryotome, mounted on a glass slide, and quickly stained with hematoxylin-eosin (H&E). A pathologist then performs microscopic analysis and provides an impression to the surgeon. Common questions include whether tumor cells are present at a surgical margin, whether any lesional tissue is consistent with an infectious or neoplastic process, and, in the latter case, whether it contains benign or malignant cells. One of the key advantages of frozen section, and microscopic examination in general, is the ability to preserve spatial variations

in the tissue. This ability is particularly important in processes such as cancer or infections that can be heterogenous or require high spatial resolution. However, there are limitations such as the turnaround time, some distortion or artifact that comes from freezing, as well as variability in the pathologic interpretation, particularly when it comes to difficult or borderline cases. Also, this technique is largely limited to H&E staining, which, although extremely valuable, cannot provide molecular, protein, or biochemical characterization, which are critical features of these disease processes. Many further details, such as tumor classification, aggressiveness, or the presence or absence of specific genomic alterations, are deferred to the evaluation of permanent section.

Permanent sections constitute a much larger fraction of pathologic specimens. To create a permanent section, the specimen is fixed in formalin, cut into representative sections, processed through a series of washing and preservation steps, and then embedded in paraffin wax. These formalin-fixed paraffin-embedded (FFPE) tissues are then cut using a microtome, mounted onto a glass slide, where the pathologist can perform a more detailed histologic analysis, which may include special stains, most notably immunohistochemical stains to determine the presence or absence of a specific protein, receptor, or other biomarker. Additional special stains are also available, including Gram, silver, acid-fast, mucicarmine, as well as many other stains that can help to detect specific pathogen types in the case of an infection being suspected. Although these FFPE specimens provide a much larger arsenal of tools, they come at the cost of time compared with frozen section, because these can take time to generate, stain, and then analyze on the order of days rather than minutes or hours. There are other areas, such as cytopathology, where fluids or cells collected by fine-needle aspiration or other means are still reviewed by pathologists using similar approaches either directly or after being preserved and reviewed as slides from cell blocks.

CLINICAL DIAGNOSTIC APPLICATIONS

Cancer Diagnostics

Cancer often leads the way in new treatment and diagnostic approaches. MSI is no exception, because many publications related to clinical MSI are cancer related. Although much of the development and optimization of MALDI MSI has been done on animal tissues, the past decade has seen a growing number of MALDI MSI studies using clinical samples. Many of these studies include FFPE or tissue microarray specimens because these tend to be the most abundantly available clinical tissues, although some have analyzed fresh or frozen tissues. Studies have included a wide variety of cancers, including, but not limited to, lung,[8–10] breast,[11–15] gastrointestinal,[8,16–22] genitourinary,[23–28] gynecologic,[29–32] renal,[33,34] and brain.[35–38] Rather than reviewing work done on each cancer type and subtype, this article discusses more global considerations and highlights specific examples where pertinent.

Tumor identification and subsequent classification remain among the primary roles of the pathologist. This process may involve identifying the presence of malignancy at frozen section or providing more detailed classification on permanent sections. The primary modalities of tumor classification involve morphologic analysis of H&E-stained sections

combined with immunohistochemistry of characteristic biomarkers, although genetic and genomic characterization have a growing role.[39] Still, each of these modalities can be time, labor, and cost intensive. In addition, there remain some gray areas or borderline cases where conventional approaches struggle to differentiate these entities and, to address these, there has been an active effort to use newer approaches such as MALDI MSI. One recent example by McDowell and colleagues[40] used MALDI MSI on FFPE specimens to differentiate pancreatic ductal adenocarcinomas based on differences in the N-glycosidase–released N-linked glycome. Gleason scoring for prostate cancer is another area where there remains diagnostically challenging cases, and Randall and colleagues[23] used MALDI MSI to characterize tumors from different Gleason grade tumors, which showed very different metabolic profiles. In another example, Calligaris and colleagues[36] used MALDI MSI on frozen samples to differentiate pituitary adenomas in as little as 30 minutes by imaging the hormone secreted by the cell of origin.

Tumor staging represents another critical element in cancer diagnostics, because focal tumors can potentially be cured by surgical resection alone, whereas cancers that have spread to lymph nodes or more distant metastatic sites generally require more aggressive or systemic treatments such as chemotherapy or radiation. Imaging modalities such as PET computed tomography or MRI can help with staging but cannot detect small numbers of cells in lymph nodes, which would change the tumor stage. Some surgical resections contain numerous lymph nodes, and currently each lymph node must be carefully reviewed by a pathologist to detect metastases and a few malignant cells could be missed. If a different modality, such as MSI, could improve sensitivity or reduce the time needed for analysis, this could help both patients as well as diagnosticians. The potential to use lipidomic signatures to detect lymph node metastases has been demonstrated for breast and thyroid tumors using DESI MSI,[41] and it stands to reason a similar approach could be used for MALDI MSI. Using a different approach, Mittal and colleagues[42] performed MALDI MSI on primary endometrial tumors and found that the likelihood to metastasize could be predicted accurately (88% tumors correctly classified) without having to look at the lymph nodes themselves. Although using such a predictive approach would be unconventional, it highlights a potential future avenue for MALDI MSI to prognosticate metastasis.

Because of its curative potential, surgical resection remains the treatment of choice for many focal tumors. Carefully determining the extent of tumor invasion is key to determining local spread. Conventional approaches use either frozen section for intraoperative guidance or assessment on permanent section to determine whether there remains any tumor at the margins of the cancer. One of the more exciting clinical MS applications is MS in the operating room as a tool for surgical guidance. By monitoring the chemical signature of the tissue, the mass spectrometer can provide faster and potentially more sensitive and accurate tumor detection. Much of the intraoperative MS work has focused on ambient MS methods because it was once thought that MALDI MSI could not be done fast enough. However, the authors have shown that it can be done in as little as 3 to 5 minutes,[43] on par with the time it takes for frozen section. Although this is not as fast as some of the intraoperative ambient approaches,[44–46] it allows clinicians to systematically cover larger areas and provides higher spatial resolution, which may be important in very heterogenous tumors or those with micrometastases.

Drug Distribution

For cancer treatment, not only are effective drugs needed but they are needed in the target tissues at appropriate concentrations. Liquid chromatography–tandem MS (LC-MS/MS) has seen increasing use in clinical chemistry laboratories, most notably in toxicology and therapeutic drug monitoring. For most drugs, there is a good correlation between circulating levels and clinical efficacy. However, for other drugs, the level in the blood may not correlate with the level in the target tissue of interest for several reasons, including host metabolism, pharmacokinetics, pharmacogenomics, and perfusion and vascularization of tissue. Subtherapeutic levels could lead to ineffective treatment as well as an environment suitable for clonal evolution and development of drug resistance. Conventionally, to determine tissue drug levels, the tissue would be weighed, homogenized, and the drug extracted before analysis. Standard curves would be performed by spiking normal tissue. Although such an approach is more widely used and vetted, homogenizing tissue leads to loss of spatial distribution of the drug. Another issue is best highlighted by a RAF inhibitor trial where the drug was shown to be effective in a murine flank model but not against intracranial implanted tumors, and the blood-brain barrier was hypothesized to be the cause.[47] Interestingly, the relative penetration of drug seemed to be adequate in both the brain and the flank. However, high-spatial-resolution MALDI MSI showed that, although the drug was perfusing the tumor, it was not diffusing into the tumor parenchyma because of thickened and abnormal vessels within the tumor, highlighting the importance of characterizing intratumor drug distribution. Additional literature reviewing progress in drug distribution by MALDI MSI can be found elsewhere.[48–50]

Infectious Disease Diagnostics

Before discussing MSI applications for infectious disease diagnostics, it is worth considering the major classes of human pathogens, namely viruses, bacteria, fungi, and parasites. Unlike cancer, where cancer cells largely share the same biochemical and metabolic pathways as other cells in the body, albeit commonly at nonphysiologic levels, most pathogens harbor distinct biochemical pathways producing distinct biomolecules, not generally produced by humans. The ability to leverage these differences based on their unique mass-to-charge ratios makes MS a powerful and agnostic method to detect pathogens in clinical specimens. Various applications to detect different classes of organisms are discussed here.

Viruses have had an outsized impact in humans over the past few decades, including annual pandemics such as influenza and emerging viruses such as Ebola, Zika, and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). When it comes to MS, the ability to uniquely distinguish pathogens relies on distinguishing the pathogens from background molecules. Because viruses do not generally have their own unique biochemical or metabolic pathways, detection of viral proteins is the most reliable and logical approach to detecting these in tissue. In most cases, viruses are detected using polymerase chain reaction (PCR)–based approaches, and the presence or absence of a virus is generally enough to guide diagnosis and management. However, there are several scenarios where tissue imaging may provide an additional benefit. One example is in neurologic infections, where inclusion bodies are seen in the tissue. Immunohistochemical staining may provide

nonspecific binding with nonviral proteins leading to false-positivity. In contrast, formalin fixation and paraffin embedding may lead to damage to the DNA or RNA, resulting in poor PCR amplification in broad-range sequencing or allele-specific PCRs. Despite MALDI MSI being available for some time, there is limited evidence of detection of viral proteins in tissue. However, it has been shown that virus-infected tissues may show different metabolic and proteomic patterns, which may help identify biomarkers of infection.[51]

Bacteria are perhaps the most ubiquitous microorganisms in both the external and internal human environments, and the proper identification and distinguishing of both commensal and pathogenic bacterial infections is critical in many patients. Although bacterial culture remains the gold standard of bacterial detection, identification, and phenotypic testing, there are several challenges that arise in the diagnostic setting. First, patients are often treated with antibiotics and, as a result, many of the organisms do not survive culture. Also, there are organisms that do not survive the preanalytical and processing steps and therefore do not grow in culture, although there may be evidence of suppurative inflammation or organisms revealed by Gram stain in tissue. Even then, it might take several days to perform these special stains. The ability to detect bacteria based on microbial signatures or specific bacterial metabolites would be of great value in the clinical setting. One example of using such an approach is presented by Juříková and colleagues,[52] where they used a rat model of *Pseudomonas aeruginosa* infection to show the presence in the lung of pyoverdine, a characteristic fluorescent siderophore and virulence factor commonly expressed in these organisms. In another approach similar to MALDI, called metal oxide laser ionization (MOLI) MS, cerium oxide can be used as the matrix to reveal unique bacterial fatty acids with the same US Food and Drug Administration (FDA)–approved MALDI-TOF MS instruments currently available in many clinical microbiology laboratories.[53] By applying this using an MSI approach in a proof-of-concept pseudoinfection model, extracted and spotted bacterial lipids were shown to be distinguishable from mouse brain lipids.[54]

Among bacteria, mycobacteria pose a particular challenge in pathology, due in part to their low organism burden in disease. For example, *Mycobacterium tuberculosis*, perhaps the most widespread infection worldwide, is particularly difficult to detect in tissue. Despite a wide area of granulomatous inflammation, often only a few organisms are detectable in tissue. For this reason, new and rapid techniques in mycobacterial detection and diagnosis in tissue specimens are helpful. Like other bacteria, mycobacterial organisms have unique biochemical products, most notably mycolic acids. Mycolic acid is not a single molecule but a group of long-chain fatty acids, which can exceed 100 carbons in length, which form a waxy membrane. This waxy membrane provides the acid-fastness, and hence their name acid-fast bacilli. High-performance liquid chromatography (HPLC) analysis of mycolic acids was once commonly used to classify different mycobacterial species, but this has largely been replaced with nucleic acid–based, or more recently MALDI-TOF–based, identification. Although MALDI-TOF classification can be used on mycobacterial isolates, there are limited studies using MSI to detect mycobacteria in tissue. One recent study by Blanc and colleagues[55] showed, in mouse and rabbit mycobacterial infection models, the presence of phosphatidyl-myo-inositol mannoside and phosphoinositol-tuberculostearic acid, which are mycobacteria-specific lipids, in the lungs of these animals. Even more importantly, the same group showed that certain

antituberculosis drugs, such as moxifloxacin, fail to penetrate the necrotizing granulomas, suggestive of potential windows or niches where the organism may survive or even evolve at subtherapeutic drug concentration.[56]

Fungi are another important class of organisms where MSI approaches may provide significant benefit. Infectious fungal pathogens, such as yeast, molds, and dimorphic molds, are a growing problem in the clinical arena, most notably in immunocompromised hosts. With the increased use of hematopoietic stem cell transplants and expansion of cellular therapies such as chimeric antigen receptor T-cell therapies, which promise to turn the tide on several hematopoietic and solid organ malignancies, the prevalence of fungal morbidity and mortality has continued to increase as more patients experience significant immunosuppression. In the context of tissue-based microbial diagnostics, there are a few aspects of fungal infections that are important to note compared with bacterial and mycobacterial disease. For one, both the relative size of fungi and their relative burden may be considerably greater than in bacterial and especially mycobacterial disease. Even the smallest yeast can be 10 to 20 times larger than bacteria. In addition, in most cases of fungal infection, the organism is apparent on histology. As such, high spatial resolution may be less of a concern. Some of the specific and commonly encountered applications include lung wedge resections, which present with evidence of fungal forms and fungal sinusitis. In both cases, the more commonly encountered organisms include *Aspergillus* species and *Mucorales* species, such as *Mucor* or *Rhizopus*. Although there are morphologic features that can distinguish these organisms, often these require more extensive special stains, such as silver staining, which can delay diagnosis. Also, the longer the organism resides in the tissue, the more atypical its histologic features can become. Therefore, the ability to use chemical signatures to distinguish these organisms may be of significant utility. Also, the speed at which this could be done would be significantly faster than nucleic acid sequencing–based approaches, which can take several days or longer, particularly because most of these must be performed at specialty reference laboratories. There is ample literature showing the ability to differentiate molds by MALDI,[57,58] so these could be applied to these tissues as well. Although there are limited applications for diagnosis of infections in human tissues, *Aspergillus* siderophores ferricrocin and triacetylfusarinine C were detected in a rat model of *Aspergillus* using MALDI FT-ICR MSI.[59] Also, like mycobacteria, the ability to characterize tissue distributions of antifungal drug levels may help guide clinical treatment of these infections.[60]

Perhaps the last, and arguably the most diverse, class of infectious pathogens is parasites, a broad class of organisms, ranging from intracellular Apicomplexa to larger multicellular organisms such as helminths, which have their own organ systems. Despite some similarities between humans and parasites, there are unique differences that could be leveraged to detect and identify these organisms in human tissue. Although there are a few examples of MSI applications to detect these organisms, this is an arguably underinvestigated field in MS, and MSI in particular, relative to its burden in humans. Perhaps the most prominent parasitic disease in terms of its public health burden in humans is malaria, which is caused by various *Plasmodium* species. *Plasmodium falciparum* is the deadliest species in humans, although there are numerous other *Plasmodium* species that infect both humans and other animals. Patterson and colleagues[61] used MALDI ionization MS to detect *Plasmodium yoelii*, which

is used in murine models, to detect infection in liver, where these organisms spend part of their life cycle. Kadesch and colleagues[62] used atmospheric pressure scanning microprobe MALDI MS to detect *Toxoplasma gondii* and *Besnoitia besnoiti* in bovine monolayer cells. Although *B besnoiti* is an important pathogen in cattle, *T gondii* is an important human pathogen in immunocompromised patients as well as pregnant hosts because of the ability of *Toxoplasma* to cross the placenta and cause fetal disease. At present *T gondii* is detected in tissues either by histologic analysis with confirmation using PCR-based or IHC-based approaches. However, either of these approaches can take considerable time, and the ability to measure these organisms quickly and rapidly would be of value. Because of their focal nature in tissue, MSI could provide a metabolic or proteomic correlate to histology.

Although clinical MSI approaches have seen an emphasis on neoplastic disease, important applications for MSI in infectious diseases are reviewed here as well. The unique and xenobiotic nature of many of these biomolecules provides an ample opportunity for both the detection and identification of pathogens. Moreover, the ability to image these molecules within tissue using MSI provides the additional advantage of mapping the pathogen within the tissue and comparing it with histologic and immunohistochemical studies, which is generally not available with nucleic acid amplification approaches. The authors suspect this will be a rich area for clinical MSI applications in the coming years.

Analytical Parameters to Consider

The variety of ionization sources and mass analyzers and combinations therein provides a multitude of analytical setups to perform MSI. Each combination provides both advantages and disadvantages, and careful consideration of the clinical diagnostic question being asked should be taken before choosing any particular platform. As with any assay, general analytical parameters such as precision, accuracy, sensitivity, selectivity, stability, matrix effects, and interferences should be considered. Beyond these, there are several additional considerations that should also be considered with MALDI MSI (and MSI in general), including tradeoffs between sensitivity and specificity, throughput and resolution, as well as cost, ease of use, and other logistical and preanalytical factors, which are discussed later. Also, it is worth noting that, although MSI is a powerful analytical approach, regardless of the ionization and mass spectrometer used, simply using an imaging approach does not always provide additional clinical diagnostic benefits. A review and some perspective on the analytical parameters to consider in the context of the clinical scenario, available specimen, and diagnostic question being asked are provided here. For clinical applications, the optimal ionization method and mass spectrometer used may be guided by the clinical question to be answered and the analytical parameters needed to answer the question. Of course, additional factors, such as availability of equipment and expertise to run and perform such assays, are critical, although these are likely to change over time.

Preanalytical Considerations

As is true with many clinical diagnostic tests, preanalytical considerations are crucially important and often overlooked. First, most pathology specimens are either frozen or FFPE specimens. If frozen, there are a few specific considerations. One is that clinical specimens are generally embedded in optimal cutting temperature (OCT) media before being mounted

and cut. Although this allows ideal sectioning in a cryostat, the OCT contains polymers that result in significant ion suppression in MALDI MSI and other MSI and MS methods. In the research setting, this can be avoided in a few ways: one is by not fully embedding the sample but by placing a small drop on which the specimen is mounted. In the clinical setting, this is very challenging, because many clinical samples are extremely small, and trying to mount and section these without embedding is impractical. Another option is using a different embedding medium that is compatible with MALDI. One study systematically tried several different embedding media and found that a supportive hydrogel was most effective and did not interfere with MALDI MSI.[63] Although using a different medium may be a long-term objective, the likelihood of changing what amounts to a nearly universal embedding/mounting medium is unlikely in the near future. Therefore, the most practical approach is removing the OCT before MSI analysis, which has been described using a technique that removes both OCT and salts, allowing better lipid analysis.[64] The other consideration is that fresh or frozen samples may still contain infectious agents, and processing poses a safety threat to people working with the specimens. Furthermore, enzymatic activity in the tissue can be reactivated during the thawing process, leading to activation of proteases, lipases, and other metabolic enzymes that will change the biochemical composition of the tissue. To avoid this, heating the tissue has been shown to both inactivate pathogens in the specimen as well as thermally inactivate the enzymes responsible for protein degradation.[65–67]

The other major specimen type is FFPE. Although the processes of formalin fixation, specimen processing, and paraffin embedding cause significant biochemical changes to the tissue, the benefit is that these specimens are biologically inactivated, and highly abundant, because they represent the largest number of specimens in most pathology departments and clinical biobanks. Despite its limitations, there has been considerable progress in processing and analysis using MALDI MSI, including enzymatic digestion by trypsin for bottom-up proteomic analysis or digestion by glycosidases for glycan analysis.[17,68–70] Regardless of whatever progress is made in working with fresh and frozen tissue, the potential to analyze the vast stores of pathology-banked FFPE tissues will continue to grow this area of development and MALDI MSI research.

Speed and Throughput Considerations

Clinical diagnostic workflows often require higher specimen throughput or shorter turnaround times than are demanded in most research settings. The considerable speed and multiplexing capacity has made MS very attractive in the clinical diagnostic space.[71] As such, assay speed is a critical feature for clinical implementation of MALDI MSI. However, when discussing speed, it is important to consider this in 2 contexts: (1) rapid workflows, and (2) high-throughput workflows.

Rapid workflows, like those needed in frozen section, where an urgent result is used for surgical guidance, the total time from sample collection to result is of utmost importance. Despite the promise of MALDI MSI, conventional methods can take an hour or more when considering matrix application and drying, image registration, and data acquisition. The authors have recently described a method to precoat and analyze a predetermined region containing the specimen with a high-frequency laser, allowing MALDI MSI, which

conventionally takes anywhere from 30 minutes to 2 hours to prepare and analyze a slide, to take less than 5 minutes.[43]

In contrast, high-throughput workflows (eg, performing MALDI MSI for all surgical pathology specimens at a hospital) may not require each individual result to be performed rapidly but, because of the total number of samples, would still require a fast process to keep up with volume. To accomplish this, high-throughput MALDI MSI workflows can leverage performing preanalytical steps in parallel. For example, if deparaffinization, trypsinization, antigen retrieval, matrix application, or recrystallization are needed, these can be done for several samples at the same time. Therefore, by aligning and automating so-called latent preanalytical steps, high-throughput analysis may be possible.

Spatial Resolution

Perhaps the most powerful and distinguishing feature of MSI compared with other MS applications is the ability to preserve spatial relationship in tissue specimens. Spatial resolution is a parameter specific to imaging applications and can be roughly defined as the ability to distinguish 2 nearby points in an image and described by pixel size. The higher the spatial resolution, the smaller the pixel size.

There are several benefits of having high spatial resolution. First, spatial preservation prevents the dilution of lesional tissue with normal, inflammatory, or otherwise nonpathogenic tissue. One example in cancer is tumor staging, where detection of malignant cells within a lymph node can change the cancer stage. The challenge lies in that there may only be rare tumor cells within the lymph node. If the lymph node was processed in aggregate, it is possible that the signal from the cancer cells would not be detected. An example in a nonneoplastic disease is the detection of rare pathogens in tissue. For example, *M tuberculosis* can have an outsized impact in tissue with large granulomatous inflammation areas with only rare organisms present. Again, if the chemical signature of an infected tissue or even a smaller dissected region of inflammation were investigated, the microbial biochemical signature could be lost in normal or inflammatory tissue signal. High spatial resolution would allow much smaller pixel size and therefore significant advantages when analyzing such organisms. Dissection approaches, such as visually circling and scraping lesional tissue or using laser microdissection, can be used to enrich tumor content. However, MSI allows analysis directly on the tissue, precluding the need to dissect the tissue. This approach both reduces the time and labor required and provides much higher spatial resolution than can is possible with physical dissection.

Another benefit of high spatial resolution is in the analysis of heterogenous tissues. Many primary tumors, for example, can be very heterogenous, containing regions of lower and higher cellularity, and admixed with stromal and immune cells. Performing conventional MS analysis on homogenized or disaggregated tissues mixes the chemical signatures of these different cells and regions, resulting in both reduced sensitivity and specificity. Being able to preserve the spatial relationships between different areas of the tissues also allows merging these analyses with other modalities, such as histology, and immunohistochemistry, providing a more comprehensive and physiologic representation of the tissue as a system.

However, one limitation, to higher spatial resolution is a relative decrease in sensitivity and increase in analytical time.

Spectral Resolution

In simplest terms, spectral resolution refers to the ability of the mass spectrometer to distinguish between 2 ions with close mass-to-charge ratio. The higher the mass spectral resolution, the more specific the mass identification. Incidentally, with the increased analytical specificity also comes increased analytical sensitivity because of a higher signal-to-noise ratio from fewer overlapping ions. There are several high-resolution MS platforms, including quadrupole TOF, Orbitrap and FT-ICR MS, the last of which can have a magnetic field as high as 21 T. There are several clinical applications in which high spectral resolution may be helpful. Most notably, most proteomic applications greatly benefit from the increased mass resolution to identify and quantify peptides. Also, this has been shown to be extremely helpful in drug analyses in tissue because of the high mass accuracy and improved signal-to-noise ratio for finding a particular drug or metabolite in tissue.

Much like spatial resolution, although it can be tempting to always want higher mass spectral resolution, there are tradeoffs as well. Perhaps the most challenging is the acquisition and maintenance of the equipment, which can be very expensive and require more technical expertise to run than lower-resolution instruments. Also, the mass resolution is only as good as its calibration and rigorous maintenance. For example, if a platform can distinguish down to 0.0001 Da, it likewise needs to be calibrated regularly to maintain this mass accuracy or it is not much more useful than a lower-resolution instrument. Note that most clinical MS that is currently performed in the clinical laboratory relies on the use of triple quadrupoles or TOF instruments, which function at lower or unit resolution. Despite this lower spectral resolution, LC-MS/MS has fundamentally transformed toxicology, therapeutic drug monitoring, inborn errors of metabolism, and other chemistry testing, and MALDI-TOF has transformed clinical microbiology microbe identification. Therefore, before opting for the highest resolving power, several issues, including the clinical need, the diagnostic question, as well as resources to both run and maintain such an instrument, must be considered.

SUMMARY AND FUTURE DIRECTIONS

As described in this article, there are numerous tissue-based clinical diagnostic applications for which MALDI MSI shows considerable promise. Not only has there been significant progress in cancer diagnostics but tissue-based infectious disease diagnostics represents another exciting area for expansion. Central to this progress will be consideration of the clinical workflows set up, and consequently which analytical parameters will be most important for the clinical diagnostic question being asked. Future applications include three-dimensional MALDI MSI, in which multiple slices of two-dimensional MALDI MSI data can be constructed to visualize another dimension of drug distribution.[72] Other areas of progress will be in drug distribution and pharmacokinetic studies, which are described in brief in this article.[47,73,74] This area is undoubtedly important in the evaluation and pharmaceutical development but may one day play a role in patient care as well. Progress in

nonneoplastic and noninfectious processes such as amyloid diagnostics is also growing.[75,76] Just as MALDI-TOF MS changed microbial diagnostics in the microbiology laboratory, the authors anticipate MALDI MSI may have a similar impact in the coming years.

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KEY POINTS

- Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) represents a powerful analytical platform for clinical tissue diagnostics.
- MALDI MSI has been moving from the basic research to the translational and clinical space.
- Clinical implementation of MALDI MSI requires consideration of the diagnostic question being addressed, clinical workflows and specimens, as well as the strengths and limitations of different platforms.

CLINICS CARE POINTS

- MALDI MSI provides comprehensive molecular images of tissue.
- Recent developments allow rapid analysis.
- Further development is needed to expand the breadth of biomolecules detected.